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KINETICS OF MACROTETROLIDE-INDUCED ION TRANSPORT ACROSS LIPID BILAYER MEMBRANES

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SUMMARY

Ion transport across lipid bilayer membranes in the presence of macrotetrolide antibiotics has been studied by stationary conductance and nonstationary relaxation methods. The results are discussed on the basis of a carrier model which has already been successfully applied to valinomycin induced ion transport. Again a kinetic analysis has been performed from which the single rate constants of the carrier model could be derived. In addition the equilibrium constant of complex formation in the aqueous phase could be determined. Measurements have been made for 4 macrotetrolides, for several ions and for various chain lengths of the lipid molecules composing the membrane.

INTRODUCTION

Some metabolic products of certain *Streptomyces* species such as valinomycin, the macrotetrolides or enniatins are able to increase the ionic permeability of biological membranes and artificial lipid bilayer membranes [1–5]. Beside this property these compounds have in common a rather specific structural peculiarity. They show a hydrophobic exterior and a hydrophilic interior. The first characteristic forms the basis for an excellent solubility in solvents of low dielectric constant such as the interior of a lipid membrane. The second property is a prerequisite for the ability of the molecules to form complexes with alkali ions in a very specific way [6–8]. Mainly because of this fact the action of these substances on the ion permeability of biological and artificial membranes has been explained on the basis of mobile carrier molecules for ions [4, 9, 10].

We have previously proposed a model for carrier-mediated ion transport through lipid membranes based on an Eyring treatment of the membrane [11]. We were able to show that this model gives a reasonable good explanation for all stationary electrical conductance data of lipid membranes in the presence of valinomycin and monactin [12]. Stationary studies alone, however, are not sufficient for a complete quantitative analysis of a transport model. But if in addition electric relaxation measurements are performed, the rate constants describing the single transport steps

may be determined. This has been demonstrated for valinomycin induced K^+ transport across negatively charged membranes [13]. Later we extended these measurements to membranes formed from neutral phosphatidylcholines with different chain lengths and for various monovalent ions in the aqueous solution [14]. For the macrotetrolide monactin and neutral lecithin membranes the amplitude of the electric relaxation process was found to be comparatively small, so that a kinetic analysis is much more difficult. These problems do not arise with membranes formed from mono- and diglycerides, as has been also suggested by Szabo and Eisenman [22]. The present paper contains a quantitative study of ion transport across monoglyceride membranes induced by the macrotetrolides nonactin, monactin, dinactin and trinactin.

MATERIALS AND METHODS

Black lipid membranes were formed from the following monoglycerides with monounsaturated fatty acids: monomyristolein, monopalmitolein, monoolein, monoeicosenoin, monoerucin and mononervonin. Subsequently they will be referred to as (14 : 1)-monoglyceride, (16 : 1)-monoglyceride, (18 : 1)-monoglyceride, (20 : 1)-monoglyceride, (22 : 1)-monoglyceride, and (24 : 1)-monoglyceride. Some experiments were performed with membranes from dioleoyl-L-α-phosphatidylcholine (di(18 : 1)-phosphatidylcholine). The monoglycerides were obtained from Nu Check Prep and from Sigma. The dioleoyllecithin was synthesized in our own laboratory [15]. The purity of the lipids was checked by thin-layer chromatography. We found only one spot on the plate in each case. Bilayer membranes were generally formed from a 0.5–1 % (w/v) lipid solution in *n*-decane. As the monoglycerides with longer hydrocarbon chains (20 : 1, 22 : 1 or 24 : 1) were not soluble in such a high concentration in *n*-decane, the mixture was briefly heated to 40 °C to obtain a clear solution.

The cell used for bilayer formation was made from Teflon and fit into a thermostated metal block as described earlier [14]. Depending on the kind of electrical measurement the circular hole in the wall between the two compartments had a diameter between 1 and 3 mm. The stationary conductance measurements were performed as described earlier [12, 14]. For the electric relaxation experiments we used a storage oscilloscope (Tectronix 5115) and a pulse generator with a rise time of 4 ns (Philips PM 5712) [14]. The macrotetrolide antibiotics were generously supplied by Ciba. Small amounts of a concentrated ethanolic solution of the antibiotics (10^{-4}–10^{-6} M) were added to the aqueous phase, to get a final concentration between 10^{-7} and 10^{-9} M. The ethanol concentration of the salt solutions generally was 0.1 % (v/v). As there was no detectable influence of the pH, we used unbuffered salt solutions with a pH of about 6. Although it had no effect on the conductivity or the current versus voltage curves, the ionic strength was kept constant at 1 M by adding LiCl. The conductance reached its maximum about 4 min after the blackening of the membrane and was independent of the membrane area within the experimental error [14]. Most of the experimental details concerning the use of the macrotetrolides correspond to those described by Szabo et al. [9].

DESCRIPTION OF THE TRANSPORT MODEL

The description and mathematical treatment of the transport model has been given in detail in previous publications [11–14]. The essential points are summarized in Fig. 1. The association-dissociation reaction between a free carrier molecule S

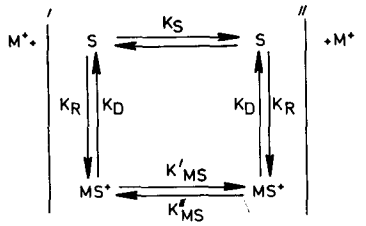


Fig. 1. Mechanism of carrier-mediated ion transport through a bilayer membrane.

from the membrane and an ion M^+ from the aqueous phase is described by the rate constants k_R and k_D. The complexes MS^+ move across the membrane with a rate constant k_{MS}, the free carrier molecule S with a rate constant k_S. The voltage dependence of k_{MS} is taken into account by an Eyring expression (Fig. 1):

$$k'_{MS} = k_{MS}\, e^{u/2}$$
$$k''_{MS} = k_{MS}\, e^{-u/2} \tag{1}$$

with the reduced voltage $u = FU/RT$ (F = Faraday constant, U = voltage, R = gas constant and T = absolute temperature). The partition coefficients (concentration ratios) for S and MS^+ between the membrane and the aqueous phase are γ_S and γ_{MS}, respectively.

Complex formation also occurs in the aqueous phase (equilibrium constant K). Complexes formed in this way may also cross the membrane (not included in Fig. 1). We have shown, however, for valinomycin and monactin that the interfacial reaction is predominant [12]. From the conductance data presented in this paper one may derive the same conclusion for the other macrotetrolides too. A similar result has been obtained by Laprade et al. [16].

The kinetic analysis is based on stationary and nonstationary electrical measurements. We have shown that from a description of the current versus voltage characteristics at different ion concentrations c_M the two independent combinations of the rate constants $z = k_{MS}/k_D$ and $v = k_{MS}k_R/k_Sk_D$ may be calculated according to the following equation [12]:

$$\frac{\lambda}{\lambda_0} = \frac{2\,(1+A)\sinh\,(u/2)}{u(1+A\cosh\,(u/2))} \tag{2}$$

with $A = 2\,z+vc_M$

The time course of the current following a voltage jump is given by:

$$J(t) = J_\infty(1+\alpha_1 e^{-t/\tau_1}+\alpha_2 e^{-t/\tau_2}) \tag{3}$$

with J_∞ = stationary current, α_1, α_1 = relaxation amplitudes and τ_1, τ_1 = relaxation times.

For the sum of the two relaxation amplitudes the following expression has been calculated [14]:

$$\alpha_1+\alpha_2 = A\cosh(u/2) \tag{4}$$

α_1, α_2, τ_1 and τ_2 depend only on the four rate constants k_R, k_D, k_{MS} and k_S, upon the ion concentration c_M in the aqueous phase and upon the applied voltage [13]. Therefore, from the knowledge of both relaxation times and amplitudes the four rate constants can be computed. If for experimental reasons only the slower relaxation time may be resolved, the analysis may be performed using the information from the stationary current versus voltage characteristic (z and v).

If the kinetic constants of transport are known, information about the equilibrium parameters of the model γ_S, K and γ_{MS} is obtained from the absolute value of the membrane conductance λ_0 at small voltages. It is given by the equation

$$\lambda_0 = \frac{F^2 d}{2RT}\frac{z\gamma_S k_R c_M c_0}{(Kc_M+1)(1+2z+vc_M)} \tag{5}$$

with d = membrane thickness, c_0 = total carrier concentration in the aqueous phase.

In principle, a measurement of the conductance λ_0 as a function of the ion concentration c_M allows a determination of γ_S and K. At small concentrations ($Kc_M \ll 1$) γ_S can be calculated. If K is not too small, λ_0 is influenced by (Kc_M+1) at high c_M and a maximum of λ_0 is observed at $c_M = [(1+2z)/vK]^{\frac{1}{2}}$. If γ_S and K are known, γ_{MS} is calculated from the relation [14]:

$$\frac{k_R}{k_D} = K\frac{\gamma_{MS}}{\gamma_S} \tag{6}$$

RESULTS AND DISCUSSION

Trinactin mediated ion transport

From the four considered macrotetrolides trinactin was found to be best suited for a quantitative kinetic analysis. Therefore most experiments were performed using this compound. A comparison between the four macrotetrolides is presented in the next section. The transport properties of trinactin were studied for different monovalent ions in the aqueous phase, for different temperatures and for various chain lengths of the lipid molecules composing the membrane. As was shown previously [9, 12] there is a linear dependence (over several orders of magnitude) of the membrane conductance upon the carrier concentration as well as upon the ion concentration in the aqueous phase. This was the reason for the assumption of a 1 : 1 complex between carrier and ion to be responsible for charge transfer across the membrane. At high ion concentration c_M the membrane conductance λ_0 may show saturation or even a maximum. This behaviour has to be expected from Eqn 5. Fig. 2 shows corresponding results for different alkali ions. With NH_4^+ a maximum in the conductance at $c_M \approx 0.3$ M is observed whereas for K^+ and Rb^+ only a saturation is visible. For Cs^+ and Na^+ λ_0 is proportional to c_M up to 1 M. According to Eqn 5 the deviation from linearity depends on the values of K and v. If z and v are known from the description of the current versus voltage characteristic (Fig. 3), K may be determined

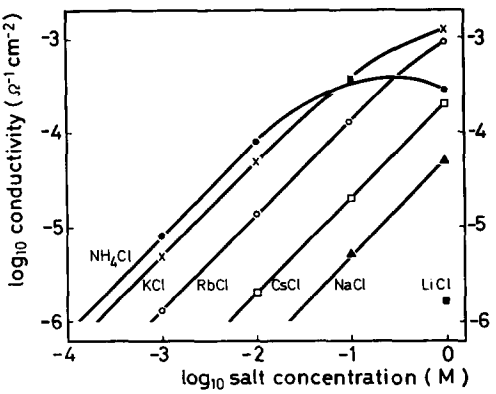


Fig. 2. Conductivity of (18 : 1)-monoglyceride membranes as a function of ion concentration in the aqueous phase for different ions. The trinactin concentration in the aqueous solution was 10^{-8} M and the temperature was kept constant at 25 °C. Except for 10^{-1} M NaCl, 10^{-2} M CsCl and 10^{-3} M RbCl, the ionic strength was held constant at 1 M by adding LiCl. The conductivity without trinactin was about $1 \cdot 10^{-7}\ \Omega^{-1} \cdot \mathrm{cm}^{-2}$. The solid lines were calculated according Eqn 5 with the experimental values given in Table II.

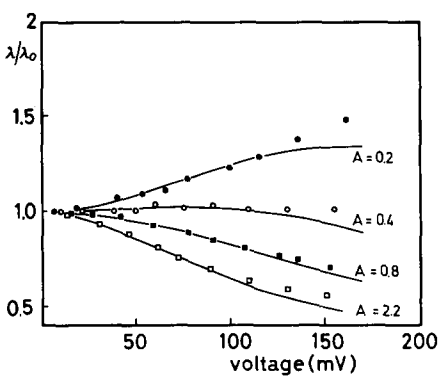


Fig. 3. Conductance ratio λ/λ_0 as a function of voltage for (18 : 1)-monoglyceride membranes and for 10^{-8} M trinactin at different salt concentrations. □, 1 M NH_4Cl; ■, 10^{-2} M NH_4Cl (+1 M LiCl); ○: 1 M RbCl; ●: 10^{-2} M RbCl (+1 M LiCl). Full lines calculated from Eqn 2 with the indicated values for A.

applying Eqn 5. Data for z and v are given in Table I. An example of their determination from the concentration dependence of λ/λ_0 (Eqn 2) is shown in Fig. 3. Table I also contains the data for the equilibrium constant K of the complex formation in the aqueous phase. K was determined using either membranes formed from (18 : 1)-monoglyceride or di(18 : 1)-phosphatidylcholine. As seen from Table I the calculated value of K is somewhat larger, if monoglyceride membranes are used. Several reasons may contribute to this comparatively small difference. One main point probably arises from the only approximative description of the voltage dependence of k_{MS} influencing the determination of z and v. k_{MS}' and k_{MS}'' have been introduced with an Eyring expression (Eqn 1). Recently on the basis of an optimum fit to steady

TABLE I

STATIONARY CURRENT VERSUS VOLTAGE AND RELAXATION DATA FOR TRINACTIN INDUCED ION TRANSPORT ACROSS (18:1)-MONOGLYCERIDE MEMBRANES AT 25 °C

Comparison between the data for different alkali metal ions. The relaxation data were measured at a salt concentration of 1 M.

Ion	z	$v[M^{-1}]$	α ($U = 60$ mV)	τ (μs) ($U = 60$ mV)	$K(M^{-1})$ (18:1)-monoglyceride	$K(M^{-1})$ Di(18:1)-phosphatidylcholine
NH_4^+	0.4	1.4	3.8	30	15	10
K^+	0.25	0.3	1.2	13	3	2
Rb^+	0.1	0.2	0.3	7.6	0.5	≤ 0.4
Cs^+	0.05	<0.05	<0.1	<4	<0.2	<0.2

state current versus voltage data modifications of the voltage dependence of the rate constants have been suggested [23, 24]. While these studies, however, depended on rather specific assumptions, a more general method of determining the voltage dependence of k_{MS}' and k_{MS}'' has now been developed. Its main result is a less strong voltage dependence of k_{MS} giving rise to a better agreement between theory and experiment (Knoll, W. and Stark, G., in preparation). Since with phosphatidylcholine membranes the quantities z and v are much smaller compared with monoglyceride membranes and therefore do hardly influence the concentration dependence of λ_0 (see Eqn 5), the data obtained with phosphatidylcholine should be more reliable. Another factor influencing the determination of K is the assumption of an equilibrium between membrane and water underlying the derivation of Eqn 5. For valinomycin and small membrane areas this condition is not given because of the rapid exchange between membrane and torus [14]. For the macrotetrolides, however, the exchange between membrane and water has been found to be faster, so that this loss is compensated at least to a large extent. Besides large membrane areas have been used in order to minimize the influence of the torus.

In conclusion the outlined procedure to determine the equilibrium constant K is still influenced by factors which are under study at present, so that the corresponding data given in Table I may be subject to minor changes, if in future finer details of the transport mechanism will be considered.

K can be determined, as described above, if it is not much smaller than 1 M^{-1}. This is only the case for NH_4^+, K^+ and Rb^+. If, however, K is known for one ion species k, it may be derived for other species i from the ratios of conductances λ_0^i/λ_0^k. For small ion concentrations $c_M(Kc_M \ll 1;\ vc_M \ll 1+2z)$ and assuming k_{MS} and γ_{MS} identical for the different ion species this ratio is given as [14]:

$$\frac{\lambda_0^i}{\lambda_0^k} = \frac{K_i\,(1+2z)_k}{K_k\,(1+2z)_i} \tag{7}$$

Using $K_{K+} = 2\ M^{-1}$ one obtains from the λ_0 data of Fig. 2: $K_{Cs+} = 6 \cdot 10^{-2}\ M^{-1}$; $K_{Na+} = 1.5 \cdot 10^{-2}\ M^{-1}$; $K_{Li+} = 5.6 \cdot 10^{-4}\ M^{-1}$.

These data clearly demonstrate that the procedure outlined has a sensitivity not yet reached by other methods. Since the equilibrium constant K for NH_4^+, K^+ and Rb^+ could be determined using Eqn 5, the application of Eqn 7 may serve as a test of consistency. From the conductance data given in Fig. 2 and the data for z and K of Table I one finds that Eqn 7 is well met for K^+ and Rb^+ (conductance ratio 0.29, right hand side of Eqn 7, 0.21). On the contrary for K^+ and NH_4^+ there is a difference of about a factor 3 between both sides of Eqn 7. This finding may be interpreted in the way that the assumption of isostericity of NH_4^+ and K^+ complexes (as required by Eqn 7) is only a rough approximation.

Recently the equilibrium constant K of complex formation in the aqueous phase has been determined by using fluorescence probes [17]. Values for NH_4^+ and K^+ have been reported which are about a factor 6 smaller than those given in Table I. These differences, however, should not be overemphasized, since the complexation properties of molecules like trinactin might be influenced by the fluorescent probes. On a qualitative basis the same series of specificity of complex formation in water has already been obtained by other methods such as salt extraction experiments [18].

Table I contains also the results of the relaxation measurements. They were obtained with 10^{-8} or 10^{-7} M trinactin in the aqueous phase. No influence of the carrier concentration up to 10^{-7} M upon the relaxation data was found. Normally the time course of the current following a voltage jump could be described by a single exponential term. It was assigned to the slower relaxation time τ_2 of Eqn 3. In some cases also the faster relaxation time could be resolved (not given in Table I). Then there is more information than needed for a kinetic analysis. It may be used for an interesting test of some of the assumptions used in the transport model (Knoll, W. and Stark, G., in preparation). For the sum of both relaxation amplitudes Eqn 4 is valid. Since z and v are known from stationary measurements, α_1 may be calculated if α_2 has been measured. For trinactin/NH_4^+ $\alpha_1 \ll \alpha_2$ was found at small voltages. If the rate constants are calculated at small voltages (see Table II), they allow the voltage dependence of α_1 and α_2 to be derived assuming Eqn 1. It is found that $\alpha_1 \ll \alpha_2$ holds for voltages up to at least 130 mV. Therefore, according to the applied vol-

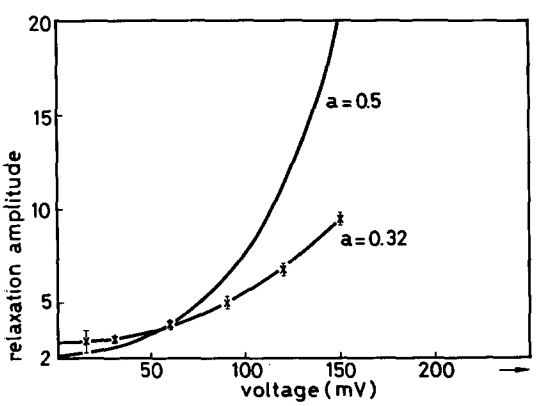


Fig. 4. Relaxation amplitude α as a function of the applied voltage. Experimental data measured with 1 M NH_4Cl and 10^{-7} M trinactin in the aqueous phase on (18 : 1)-monoglyceride membranes and 25 °C. Full lines calculated from the theory with the indicated values for a. See also text.

tage dependence of k_{MS}' and k_{MS}'', α_2 should be proportional to cosh $(u/2)$ (Eqn 4). As is shown in Fig. 4, the experimental data can be better fitted assuming $a \cdot u$ instead of 0.5 u (with $a = 0.32$). This finding supports the conclusion that the hitherto used voltage dependence of k_{MS}' and k_{MS}'' is only approximate. This is also one reason for the deviations from theory in the description of current versus voltage characteristics observed at higher voltages (Fig. 3), as has been reported earlier [12, 19]. This problem will be discussed in more detail in a forthcoming publication. In the present paper the voltage dependence according the Eqn 1 has been used, since the modified voltage dependence changes the rate constants only in a rather small extend.

A further test of consistency is the measurement of the concentration dependence of the relaxation data. For trinactin/NH_4^+ the relaxation process could be resolved down to NH_4^+ concentrations of 10^{-2} M. A satisfactory agreement between theory and experiments was obtained (e.g. a relaxation amplitude $\alpha = 1.4$ is calculated from the rate constants given in Table II at 10^{-2} M, which has to be compared with an experimental value of 1.7 at 60 mV). Table II contains the values of the rate

TABLE II

RATE CONSTANTS AND PARTITION COEFFICIENTS FOR TRINACTIN-INDUCED ION TRANSPORT ACROSS (18:1)-MONOGLYCERIDE MEMBRANES AT 25 °C

The data were calculated from the experimental values given in Table I and Fig. 2.

Ion	k_R ($M^{-1} \cdot s^{-1}$)	k_D (s^{-1})	k_{MS} (s^{-1})	k_S (s^{-1})	γ_S	γ_{MS}
NH_4^+	$1.9 \cdot 10^5$*	$2 \cdot 10^4$	$8 \cdot 10^3$	$5.4 \cdot 10^4$*	$3 \cdot 10^4$*	$1.5 \cdot 10^4$
K^+	$6.5 \cdot 10^4$*	$6.4 \cdot 10^4$	$1.6 \cdot 10^4$	$5.4 \cdot 10^4$*	$4.8 \cdot 10^4$*	$1.6 \cdot 10^4$
Rb^+	$1.1 \cdot 10^5$	$1.6 \cdot 10^5$**	$1.6 \cdot 10^4$**	$5.4 \cdot 10^4$	$1.7 \cdot 10^4$	$2.7 \cdot 10^4$

* These data were calculated using k_S derived from the Rb^+ data.
** Calculated assuming $k_{MS}(Rb^+) = k_{MS}(K^+)$ (see text).

constants and of the partition coefficients γ_S and γ_{MS} for different ions. They were calculated from the experimental data given in Table I. As has been pointed out in a previous paper [14], in many cases only two of the four rate constants may be calculated with reasonable accuracy. If the measured relaxation amplitude fulfills the condition $\alpha \ll (2z+vc_M) \cosh(u/2)$, then k_R and k_S are obtained with a relatively small error, whereas k_D and k_{MS} can change many orders of magnitude within the scatter of the experimental data. If on the other hand α is close to $(2z+vc_M)\cosh(u/2)$, k_D and k_{MS} may be determined. Only within a limited range of values of α in comparision to $(2z+vc_M)$, it is possible to determine all four rate constants. For NH_4^+ and K^+ only k_D and k_{MS} could be derived. For these ions k_R and k_S had to be determined in another way. Since k_S does not depend on the kind of the transported ion, it may be taken from the Rb^+ data. Then k_R is obtained from the quantity v. Nevertheless the data for k_R (and k_S) are subject to a greater error than those for k_D and k_{MS}. On contrary for Rb^+ only k_R and k_S could be calculated using the normal procedure. In this case k_{MS} from the K^+ analysis was used (assuming isostericity), while then k_D was obtained from the quantity z. The values of k_R for trinactin and monoglyceride membranes given in Table II are about 5 times larger than those obtained for valinomycin

and phosphatidylcholine membranes of equal chain length of the lipid molecules [14]. A true comparison, however, is not possible, since k_R was found to depend rather strongly on structural properties of the membrane. Whereas k_R is similar for different ions, there is an increase in the dissociation rate constant k_D in the series NH_4^+, K^+ and Rb^+ reflecting a decreasing stability constant $K_h = k_R/k_D$. It may be interesting to note that the K_h data for NH_4^+ and K^+ agree well with results obtained by Kemp and Wenner [25] studying complex formation of trinactin monolayers [25]. The translocation rate constants k_S and k_{MS} for the free and the complexed carrier molecules differ by about a factor of 5. This may be caused by a somewhat different energy barrier for diffusion across the membrane. The values for the partition coefficients of the free carrier molecules γ_S should not depend on the kind of ion. A mean value of $2.9 \cdot 10^4$ is calculated (assuming a membrane thickness of 50 Å). The scatter of the data indicates the error of the procedure. Since in the evaluation of γ_{MS} additional sources of error may play a role (errors in K), the differences for the various ions may not be significant. A similar comparison between trinactin induced transport of different ions has been performed by Laprade et al. [16] using membranes formed from glyceryldioleate.

Table III contains experimental data for the system trinactin/NH_4^+ and (18 : 1) monoglyceride membranes at different temperature. As has already been discussed above, only k_D and k_{MS} could be obtained (Fig. 5). In both cases an activation energy of about 20 kcal/mol may be estimated. Because of the uncertainty

TABLE III

STATIONARY CURRENT VERSUS VOLTAGE AND RELAXATION DATA FOR MEMBRANES FORMED FROM (18:1)-MONOGLYCERIDE MEMBRANES AT DIFFERENT TEMPERATURES IN THE PRESENCE OF TRINACTIN AND NH_4^+

K was derived from measurements on di(18:1)-phosphatidylcholine membranes. The stationary conductance λ_0 was measured with 10^{-8} M trinactin in the aqueous phase using (18:1) -monoglyceride membranes. For relaxation measurements c_M was 1 M.

Temperature (° C)	z	$v(M^{-1})$	α ($U = 60$ mV)	τ (μs) ($U = 60$ mV)	$K(M^{-1})$	λ_0 ($\Omega^{-1} \cdot cm^{-2}$)	
						$c_M = 1$ M	$c_M = 10^{-2}$ M
5	0.4	3.2	7	210	17	$7.0 \cdot 10^{-5}$	$4.5 \cdot 10^{-5}$
25	0.4	1.4	3.8	30	10	$2.7 \cdot 10^{-4}$	$8 \cdot 10^{-5}$
40	0.35	1.1	2.5	5.5	7	$2.6 \cdot 10^{-3}$	$2.5 \cdot 10^{-4}$

in k_R, the temperature dependence of the partition coefficient γ_S is not given. It is substantially smaller than for valinomycin [14]. This finding is in agreement with results obtained from temperature jump measurements with monactin (Stark, G., unpublished). This method allows the separation of different temperature dependent processes such as the partition of the carrier between membrane and water and the kinetic constants of ion transport [19]. The equilibrium constant K for complex formation between trinactin and NH_4^+ in the aqueous phase decreases with increasing temperature. This was found with experiments on monoglyceride and phosphatidylcholine membranes. From the values given in Table III one can calculate a reaction

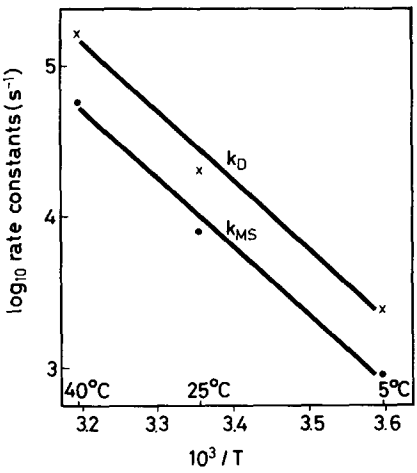


Fig. 5. Temperature dependence of the dissociation rate constant k_D and the translocation rate constant k_{MS} for (18 : 1)-monoglyceride membranes, trinactin and NH_4^+.

enthalpy ΔH of about -5 kcal/mol. For monactin/Na^+ in methanol $\Delta H = -6.6$ kcal/mol has been found [20].

In a previous publication we have shown that the kinetic constants of valinomycin induced ion transport depend on the chain length of the lipid molecules composing the membrane [14]. Similar results have been obtained with trinactin as a carrier. As shown in Table IV the chain lengths of the monoglycerides were varied from 14 to 24 carbon atoms and the transport parameters in the presence of NH_4^+ were measured. A decrease of z, v and λ_0 with increasing chain length was observed, whereas the relaxation time τ showed an opposite behaviour. For reasons which are not known at present the data for the longest chains (24 : 1) deviate somewhat from this general

TABLE IV

STATIONARY CURRENT VERSUS VOLTAGE AND RELAXATION DATA FOR MEMBRANES FORMED FROM MONOGLYCERIDES WITH DIFFERENT CHAIN LENGTHS IN THE PRESENCE OF TRINACTIN AND NH_4^+

λ_0 was measured with 10^{-8} M trinactin in the aqueous phase, whereas the relaxation experiments were performed with 1 M NH_4Cl. The temperature was kept constant at 25 °C.

Chain-length of monoglycerides	z	$v(M^{-1})$	α ($U = 25$ mV)	τ (μs) ($U = 25$ mV)	λ_0 ($\Omega^{-1} \cdot cm^{-2}$)	
					$c_M = 1$ M	$c_M = 10^{-2}$ M
14:1	2.5	15*	20	5.5	$5.5 \cdot 10^{-4}$	$2.7 \cdot 10^{-4}$
16:1	2	5*	8.5	15	$5.0 \cdot 10^{-4}$	$1.5 \cdot 10^{-4}$
18:1	0.4	1.4	2.8	35	$2.7 \cdot 10^{-4}$	$8 \cdot 10^{-5}$
20:1	0.3	1.2	1.35	58	$2.3 \cdot 10^{-4}$	$6 \cdot 10^{-5}$
22:1	0.22	0.46	0.57	155	$3.6 \cdot 10^{-4}$	$8 \cdot 10^{-5}$
24:1	0.17	0.66	0.85	108	$1.4 \cdot 10^{-4}$	$3 \cdot 10^{-5}$

* These values for v were derived from the concentration dependence of λ_0 (Eqn 5), assuming $K = 15\ M^{-1}$.

TABLE V

KINETIC AND EQUILIBRIUM CONSTANTS OF TRINACTIN INDUCED NH_4^+ TRANSPORT ACROSS MEMBRANES FORMED FROM MONOGLYCERIDES WITH DIFFERENT CHAIN LENGTHS AT 25 °C

γ_{MS} was calculated assuming $K = 15\ M^{-1}$. The data were calculated from the experimental values given in Table IV.

Chain-length of mono-glycerides	$k_R(M^{-1} \cdot s^{-1})$	$k_D(s^{-1})$	$k_{MS}(s^{-1})$	$k_S(s^{-1})$	γ_S	γ_{MS}
14:1		$3.5 \cdot 10^4$	$8.9 \cdot 10^4$			$1.5 \cdot 10^4$
16:1		$1.6 \cdot 10^4$	$3.2 \cdot 10^4$			$1.9 \cdot 10^4$
18:1	$1.9 \cdot 10^5$★	$2 \cdot 10^4$	$8 \cdot 10^3$	$5.4 \cdot 10^4$★	$2.3 \cdot 10^4$★	$1.5 \cdot 10^4$
20:1	$2.5 \cdot 10^4$	$4 \cdot 10^4$	$1.2 \cdot 10^4$	$6.2 \cdot 10^3$	$1.6 \cdot 10^5$	$6.5 \cdot 10^3$
22:1	$6 \cdot 10^3$	$1.8 \cdot 10^4$	$4 \cdot 10^3$	$2.9 \cdot 10^3$	$1.1 \cdot 10^6$	$2.4 \cdot 10^4$
24:1	$1.6 \cdot 10^4$	$2.8 \cdot 10^4$	$4.7 \cdot 10^3$	$4.2 \cdot 10^3$	$1.8 \cdot 10^5$	$6.9 \cdot 10^3$

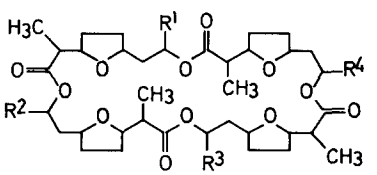
★ For k_R, k_S and γ_S on (18:1)-monoglyceride membranes see Table II and text.

tendency. The rate constants are given in Table V. k_R and k_S could only be determined for membranes formed from monoglycerides with chain lengths longer than 18 carbon atoms. As in the case of valinomycin, k_R and k_S decrease with increasing chain length. For valinomycin the strong variation in k_R has been explained on the basis of a chain length dependent microviscosity of the membrane, which could influence the association rate in different ways [14]. The fact that this effect is now also observed for another carrier (trinactin) supports the given interpretation. Also the chain length dependence of the translocation rate k_S is similar for both carrier systems and for both kinds of membranes. In contrast to valinomycin the translocation rate constant k_{MS} could be determined for trinactin. It was found that the chain length dependence of k_{MS} and k_S agree qualitatively. Their reduction with increasing chain length may also result from a variable fluidity of the membrane and/or the increasing membrane thickness. For membranes formed from monoglycerides dissolved in n-decane the membrane thickness was found to be proportional to the chain length of the lipid molecules (Benz, R., unpublished). While k_R as well as k_{MS} and k_S are rather strongly influenced by structural properties of the membrane, the dissociation rate constant k_D is not influenced by the chain length of the lipid molecules (mean value $2.5 \cdot 10^4\ s^{-1}$). It seems to depend only on molecular properties of the complex.

The partition coefficient γ_{MS} for the complex also proved to be independent of the chain length of the lipid molecules within the experimental error (mean value $1.4 \cdot 10^4$). On the other hand the partition coefficient γ_S for the free carrier increases for longer chain lengths. This difference may arise from the somewhat different location of both species at the interface. The complex should have a more hydrophobic exterior and may be located near to the hydrophobic side of the membrane interface, whereas the free carrier molecules may be situated more close to the polar headgroups of the membrane surface. It might therefore react much more sensitively to small changes of the properties or structure of the interface as a consequence of an increase of the chain length of the lipid molecules.

Comparison of different macrotetrolides

The four macrotetrolides used throughout the present study differ only in the number of ethylgroups (Fig. 6). In the series nonactin, monactin, dinactin and trinactin methyl groups are consecutively replaced. Despite these rather small differences in structure, influences on the complexation properties in bulk phases as well as on salt extraction into organic solvents have been observed [7, 21]. The conductance of bilayer membranes induced by macrotetrolides was found to increase from nonactin up to trinactin [9]. In the frame of the present study we have tried to analyse the slightly varying transport properties with respect to differences in the rate constants.



$R^1 = R^2 = R^3 = R^4 = CH_3$ NONACTIN
$R^1 = R^2 = R^3 = CH_3$ $R^4 = C_2H_5$ MONACTIN
$R^1 = R^3 = CH_3$ $R^2 = R^4 = C_2H_5$ DINACTIN
$R^1 = CH_3$ $R^2 = R^3 = R^4 = C_2H_5$ TRINACTIN

Fig. 6. Structure of the macrotetrolides.

NH_4^+ has been used as transported ion, since it shows the largest relaxation amplitude. The experimental data are summarized in Fig. 7 and Table VI. The equilibrium constant K for complex formation in water has been found to increase from nonactin to trinactin by a factor of about 30. This is concluded from the variation of saturation behaviour of λ_0 at high salt concentrations. In contrast to K the quantities, z, v, α and τ do not change very much. This is also reflected in the values for the single rate constants presented in Table VII. Therefore we have to conclude that the main difference between the four macrotetrolides consists in the equilibrium constant of complex formation in the aqueous phase.

TABLE VI

STATIONARY CURRENT VERSUS VOLTAGE AND RELAXATION DATA FOR THE NH_4^+ TRANSPORT ACROSS (18:1)-MONOGLYCERIDE MEMBRANES INDUCED BY THE DIFFERENT MACROTETROLIDES AT 25 °C

The relaxation experiments were performed at 1 M NH_4Cl.

Macrotetrolide	z	$v(M^{-1})$	α ($U = 60$ mV)	τ (μs) ($U = 60$ mV)	$K(M^{-1})$	
					(18:1)-monoglycerides	di-(18:1)-phosphatidylcholine
Nonactin	0.15	0.3	0.84	35	0.5	$\leq$0.4
Monactin	0.3	0.5	1.3	39	1	0.9
Dinactin	0.4	0.8	2.1	38	6	4
Trinactin	0.4	1.4	3.8	30	15	10

TABLE VII

KINETIC AND EQUILIBRIUM CONSTANTS FOR MACROTETROLIDE INDUCED NH_4^+-TRANSPORT ACROSS (18:1)-MONOGLYCERIDE MEMBRANES AT 25 °C

The values were calculated from the experimental data given in Table VI and Fig. 7.

Macrotetrolide	$k_R(M^{-1} \cdot s^{-1})$	$k_D(s^{-1})$	$k_{MS}(s^{-1})$	$k_S(s^{-1})$	γ_S	γ_{MS}
Nonactin	$3.4 \cdot 10^4$	$4.3 \cdot 10^4$	$6.5 \cdot 10^3$	$1.7 \cdot 10^4$	$4.1 \cdot 10^4$	$6.4 \cdot 10^4$
Monactin	$2 \cdot 10^4$	$2.5 \cdot 10^4$	$7.6 \cdot 10^3$	$1.2 \cdot 10^4$	$7.9 \cdot 10^4$	$6.4 \cdot 10^4$
Dinactin	$2.5 \cdot 10^4$	$2 \cdot 10^4$	$8 \cdot 10^3$	$1.2 \cdot 10^4$	$7.3 \cdot 10^4$	$1.5 \cdot 10^4$
Trinactin	$1.9 \cdot 10^5$ *	$2 \cdot 10^4$	$8 \cdot 10^3$	$5.4 \cdot 10^4$ *	$2.3 \cdot 10^4$ *	$1.5 \cdot 10^4$

* k_R, k_S and γ_S for trinactin see Table II and text.

A somewhat surprising result is the small decrease of γ_{MS} in the series nonactin to trinactin. If only hydrophobic interactions are taken into account γ_{MS} should be largest for trinactin. Assuming a contribution of about 500 cal/mol for each CH_2-group, the differences of the free energy between membrane and water should be 1.5 kcal/mol larger for trinactin compared with nonactin (corresponding to γ_{MS} (trinactin)/γ_{MS}(nonactin) $\approx$ 13). This is not in agreement with the data given in

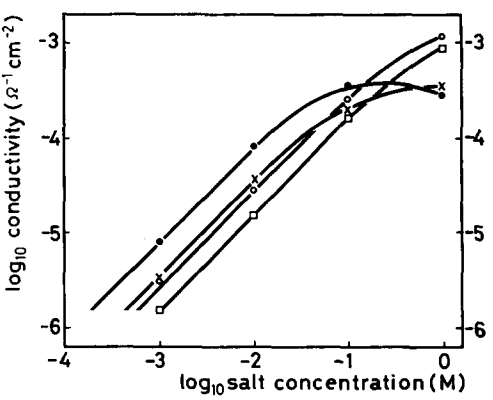


Fig. 7. Conductivity of (18 : 1)-monoglyceride membranes for different macrotetrolides as a function of the NH_4Cl concentration in the aqueous phase at small voltages and 25 °C. Except for 10^{-3} M NH_4Cl, (in case of nonactin and monactin) the ionic strength was held constant at 1 M by adding LiCl. The conductivity without any macrotetrolide was about $10^{-7}\ \Omega^{-1} \cdot cm^{-2}$. The solid lines were calculated according Eqn 5 with the data given in Table VI. ●, 10^{-8} M trinactin; ×, 10^{-8} M dinactin; ○, 10^{-8} M monactin; □, 10^{-8} M nonactin.

Table VII. One may conclude therefore, that either the above theoretical consideration is not valid for molecules with a size such as nonactin, or that the partition coefficients between membrane and water cannot be calculated merely on the basis of a rather gross treatment of the membrane as a hydrocarbon bulk phase. Then in addition further molecular interactions must be considered arising from special properties of a membrane interface.

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